

A preliminary report on newborn screening of inborn metabolic disorders

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ABSTRACT

Background: Newborn screening (NBS) for metabolic and genetic disorders, which can be treated or modified if detected early in life, can help to prevent potentially disastrous consequences and save a precious life. However, there is no nationwide consensus on neonatal screening in India; hence, several treatable cases may be missed. **Objective:** The objective of this study was to detect the prevalence of selected metabolic disorders among neonates and to diagnose them as early as possible to minimize the morbidity and mortality. **Materials and Methods:** This prospective study included all live newborns delivered during period 2015–2016 in a tertiary care neonatal unit in Kolkata. On the 3rd day of life, newborns were subjected to detailed history, with special emphasis on family history and history of consanguinity. This was followed by thorough clinical examination and metabolic screening for congenital hypothyroidism (CH), G6PD deficiency, phenylketonuria (PKU), congenital adrenal hyperplasia (CAH), galactosemia, and cystic fibrosis with heel prick blood sample collected on blotting paper. **Results:** Of 1373 babies, four babies were screened positive of CH, G6PD deficiency, CAH, and PKU, respectively. However, confirmatory test for PKU came out to be negative. **Conclusion:** NBS can be an important preventive public health program. Application of tandem mass spectrometry to newborn screening for metabolic disorders offers rapid results and covers a wide range of disorders. However, the screen-positive tests need to be confirmed by actual enzyme assay (for deficiency), chromatography/mass spectroscopy analysis, or DNA testing.

Key words: Congenital adrenal hyperplasia, Congenital hypothyroidism, G6PD deficiency, Inborn errors of metabolism, Newborn screening, Tandem mass spectrometry

Inborn errors of metabolisms (IEMs) are a complex and heterogeneous group of monogenic disorders. It exhibits clinical symptoms due to an error in a genetic code, resulting in a lowered or deficient activity of an enzyme in a single pathway of intermediary metabolism [1]. Delay in the diagnosis and treatment of these disorders leads to a variety of adverse outcomes such as moderate-to-severe neuropsychological dysfunction, mental retardation, and even death. Early and timely diagnosis and intervention of these disorders can modify the course of disease and, hence, significant morbidity or mortality. Each disorder is individually rare, but their cumulative incidence is relatively high, around 1 in 1500–1 in 5000 live births [2,3].

Newborn screening identifies apparently healthy infants with serious inherited disorders. For metabolic disorders, the National Neonatology Forum, India, recommends that newborn screening (NBS) for three disorders - congenital hypothyroidism (CH), congenital adrenal hyperplasia (CAH), and G6PD deficiency - should be done in all babies [4]. It can be done by enzymatic assay for enzyme deficiency. Tandem mass spectrometry (TMS) can be used for rapid identification and quantification of a large number of different analytes from a single sample. Most of the data suggest that the samples be taken between 2 and 7 days of age. In the case of screening

for CH, the huge variations in the levels of thyroid-stimulating hormone (TSH) in the first 48 h after birth make it sensible to do a TSH screening only after 48–72 h of birth. Other variables such as prematurity, blood transfusion, and parenteral nutrition also influence the timing of newborn screening. Early screening enables results to be obtained by 2 weeks of age, and the baby could be started on specific therapy or special elimination diets before morbidity sets in. The cord blood sample cannot be used to screen for metabolic disorders, as for their manifestation some feeding is required. IEMs cannot be detected biochemically until at least 12 h after the baby has taken feeds.

Natural history and the prevalence of each metabolic disorder vary from country to country, region to region, and also in different ethnic groups. Therefore, the existing data and studies cannot be applied to whole India. Preventing these disorders will be more useful than trying to let these disabilities develop and then treat.

MATERIALS AND METHODS

This prospective study was conducted during the period of 2015–2016, in a tertiary care neonatal unit at Kolkata, India. After getting the ethical committee approval, all the live born neonates who were born in the hospital, irrespective of their gestational age

and birth weight, were included in the study. We obtained consent from the respective parents. The babies who were not initiated feeding before 3 days and were on parenteral nutrition since birth were excluded from the study.

On the 3rd day of life, newborns were subjected to detailed birth history, thorough clinical examination, with special emphasis on family history and history of consanguinity, and also metabolic screening for CH, G6PD deficiency, phenylketonuria (PKU), CAH, galactosemia, and cystic fibrosis. Sample consisted of heel prick blood sample collected and air dried on blotting paper. The dried sample was sent for ELISA-based enzymatic assay and TMS. Cost of the above-mentioned metabolic screening test was Rs. 500 and hospital bores the cost of these investigations.

RESULTS

A total number of 1373 newborns were screened; of which, four infants were screened positive including one each for CH, G6PD deficiency, CAH, and PKU, respectively. However, confirmatory test for PKU came out to be negative, and therefore, the incidence came out to be 1:1373 for CH, CAH, and G6PD deficiency each.

The newborn screened positive for CH, which was confirmed (table 1) serum TSH and T4 levels and was immediately commenced on thyroxin replacement therapy of 10–15 mcg/kg/day. Baby is on regular follow-up, diagnosed to have permanent CH, and is on thyroxin replacement therapy. There was no maternal history of any thyroid disorder. Similarly, the male baby screened positive for G6PD deficiency also presented with neonatal hyperbilirubinemia and was confirmed by the analysis of enzyme activity. Baby is under regular follow-up, and there has not been any further episode of hyperbilirubinemia.

One male baby screened positive for CAH was confirmed by serum 17-OH progesterone level and was started glucocorticoid replacement therapy. There was no episode of salt-wasting crisis, and the baby was referred to a higher center with pediatric endocrinology department for further evaluation, management, and counseling. All above-mentioned four cases had no prior positive family history while the history of consanguinity was present in baby with G6PD deficiency.

DISCUSSION

Newborn screening identifies apparently healthy infants with serious inherited disorders, generally metabolic in origin, that are

usually correctable by dietary or drug interventions before they suffer significant morbidity or mortality. It is originated with the work of Robert Guthrie, “Guthrie test,” in the 1960s, for detecting the metabolic disorder, PKU, and used for years to justify screening programs. In expanded newborn screening, a single test allows for early detection and treatment of a large number of disorders, and it can potentially prevent serious consequences [5].

The ICMR initiated a program to screen neonates in five centres in India - Delhi, Mumbai, Chennai, Hyderabad, and Kolkata. The study lasted for 5 years from 2007 to 2012 where the overall numbers of newborns enrolled for screening were 103,849 for CH and 103,712 for CAH. As compared to our study where the incidence of CH was 1:1373, the incidence of CH overall was 1 in 1130 newborns, varying from 1 in 727 in Chennai to 1 in 1528 in Mumbai. Similarly, as compared to the incidence of CAH which was 1:1373 in our study, the overall incidence of CAH was 1 in 5762 newborns, varying from 1 in 2036 in Chennai to 1 in 9983 in Mumbai. Apart from the incidence rates, the ICMR study also provides good information on the actual process of screening and how to set up such a program [6]. As per Shriram *et al.* [7], it was 1 in 2000 newborns in Chennai where 30,514 babies were involved in the study. It was observed that the incidence of CAH was higher in southern states.

In our study, the incidence of G6PD deficiency came out to be 1 in 1373 (0.07%), whereas it was 0.89% as per the study of Kaur *et al.* [8] and 2% as per Pao *et al.* [9]. Many countries such as the United States do not advocate G6PD deficiency screening in their NBS program. The reason is that the appearance of jaundice alerts one to obtain assay of G6PD enzymes, so a deficient person would be detected even if newborn screening was not done. It must be stated, however, that, in the presence of acute hemolysis due to the presence of some fresh red blood cells, the value of G6PD enzyme may appear to be falsely normal.

The incidence of CH appears increasing over the past 20 years. Whether the increase is real or is it the result of changes in the racial/ethnic population or lowering of screening test cut-offs, or it may be due to an increase in preterm births. There is also uncertainty concerning permanent versus transient CH during monitoring. We need studies in affected infants detected by abnormalities on a second screening test, to confirm whether or not these infants have transient or permanent hypothyroidism.

The World Health Organization “Wilson–Jungner” criteria are used to determine the disorders that need to be included in the NBS program. According to it, a disease which has the following

Table 1: Babies screened and number of positive cases

Clinical condition screened	Number of babies screened	Number of cases positive for NBS	Confirmatory test (positive/negative)
CH	1373	01	Positive
Galactosemia	1373	00	-
G6PD deficiency	1373	01	Positive
PKU	1373	01	Negative
CF	1373	00	-
CAH	1373	01	Positive

PKU: Phenylketonuria, CH: Congenital hypothyroidism, CF: Cystic fibrosis, CAH: Congenital adrenal hyperplasia

properties should be screened: (1) An important health problem; (2) the natural history of the condition should be adequately understood; (3) it should be recognizable in the early stages; (4) there should be a suitable test or examination; (5) the test should be acceptable to the population; (6) intervals for repeating the test should be determined; (7) there should be an accepted treatment; (8) facilities for diagnosis and treatment should be available; (9) there should be an agreed policy concerning whom to treat as patients; and (10) the costs of case finding should be economically balanced against the benefits [10].

An NBS program is not just a panel of screening tests, and it also integrates five parts as follows: (1) Testing of newborn infants, (2) follow-up of an abnormal screening result, (3) diagnostic testing and confirmation by specialized laboratory testing, interpretation, and treatment, (4) lifelong disease management, and (5) the NBS system's evaluation, which makes regular and timely communication between nurseries, screening laboratories, health authorities, pediatricians, and subspecialists [11].

TMS is a powerful technology used for rapid identification and quantification of a large number of different analytes from a single sample, by separating the ions based on their molecular mass-to-charge ratio and measuring their intensities. The application of TMS to newborn screening currently involves measuring mainly two groups of analytes, amino acids and acylcarnitine species, for the detection of aminoacidopathies, organic acidurias, and fatty acid oxidation defects. Advantages of using TMS for NBS include the following: (a) The analysis can be performed on very small quantities of blood or other body fluids; (b) the analysis uses two mass spectrometers concurrently. The first is used to separate the components of the mixture, hence eliminating or minimizing prior chromatographic separation; (c) the time required for the analysis is minimal; and (d) the process can be automated, permitting analysis of around 600 samples per 24 h, with a very modest cost per sample [11].

Newborn screening, as a routine part of the care of most newborns, is expanding considerably throughout the world. Many countries screen all newborns for PKU and hypothyroidism, while in some of the more developed countries, the routine screening panels include 20 or more conditions [12]. As per the American College of Medical Genetics recommendations, 29 core conditions that are detectable by TMS are considered appropriate for newborn screening [11].

The expanded newborn screening using TMS for 30–40 inherited IEMs can be offered to the “well to do” families and strong family history of metabolic disorders. The incidence of CAH is higher in the southern states than in the northern states. This is another strong argument for initiating NBS in India [11–14]. Based on our study, we will recommend NBS for CH, CAH, and G6PD deficiency, and for CH, it should be included the nationwide program.

Our study also had a limitation as this being a hospital-based study and sample size being small; the incidence rate may not reflect what is in the community. At the hospital, the measurements were done by a single investigator which may not

be the same at community level where multiple health workers are involved. In this study, we did not test the usefulness of these tests after 4th day of life.

CONCLUSION

To minimize morbidity, mortality, and disabilities associated with the inherited metabolic disorders, we suggest a national pilot study, to evaluate the establishment of the expanded NBS in this country. Based on current evidence, all hospitals in urban areas in India should initiate NBS for CH, CAH, and G6PD deficiency at nominal cost (Rs. 500 in our study). Facilities for confirmation of diagnosis follow-up and treatment should be established. For a nationwide program, screening for CH should be set up.

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